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Title: TRACERS AND ASSEMBLY FOR LABELING CHEMICAL OR BIOLOGICAL MOLECULES METHODS AND KITS USING THE SAME

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Claim 22 (cancelled)

Claim 23 (cancelled)

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Claim 25 (cancelled)

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Claim 27 (cancelled)

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Claim 29 (cancelled)

Claim 30 (cancelled)

Claim 31 (cancelled)

Claim 32 (cancelled)

Claim 33 (cancelled)

Claim 34 (cancelled)

Claim 35 (cancelled)

Claim 36 (cancelled)

Claim 37 (cancelled)

Claim 38 (original): A process for generating novel enhanced shape encoding particles (SEP's) which maximizes particle encoding capacity and reserves a centralized probe attachment zone, comprising the steps of:

fabricating a planar poly-layered silicone wafer flake further comprised of a first micron-scale thickness polycrystalline Si layer disposed upon a bottom thinner dissolvable layer of SiO₂:

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photolithographically etching with reactive ions desired geometric shape outlines

in the top Si layer;

releasing resulting shaped flakes by dissolving the bottom dissolvable layer of

SiO₂ with HF acid whereby said desired geometric shape outlines further comprise peripheral

notches extending not more than 1/6 th of the diameter of the resulting shaped flakes into a

central region of the resulting shaped flakes.

Claim 39 (cancelled)

Claim 40 (original): A product produced by the process of claim 38.

Claim 41 (original): In a process for encoding the identity of microscopic particles by

shape, wherein such particles can be combined in large numbers while remaining readily

distinguishable and having their individual identities recovered, the improvement comprising:

fabricating a multiplicity of shape encoded particles (SEP's) each having a top

surface and a bottom surface and each having a unique geometric configuration wherein each of

said multiplicity of SEP's is effective for being readily arranged such that each of the top and

bottom surfaces rests in a plane parallel to a plane of a surface upon which the bottom surface is

disposed enabling display of a definitive outline when imaged by a suitable technique.

Claim 42 (original): A novel enhanced SEP product, produced by the process of claim

41, wherein a definitive outline enables identification of the SEP and any material previously

associated with the SEP through at least one of binding and contact with the SEP.

Claim 43 (original): A novel enhanced SEP product, produced by the process of claim

41, having a dimensional range extending from the nanometer to the millimeter scale.

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Claim 44 (currently amended): A novel enhanced SEP product, produced by the

process of claim 41, wherein shape detection is accomplished by at least one imaging technique

selected from the group consisting of optical microscopic imaging, near field microscopy,

electron microscopy, scanning-tunneling microscopy and atomic force microscopy.

Claim 45 (original): A novel enhanced SEP product, produced by the process of claim

41, wherein resolution of the definitive outline of said SEP is facilitated by automated image

recognition software.

Claim 46 (original): A novel enhanced SEP product, produced by the process of claim

41, wherein the SEP has a diameter of less at least about three microns and said unique

geometric configuration is less than the wavelength of visible light.

Claim 47 (original): A novel enhanced SEP product, produced by the process of claim

41, effective to transport organic matter bound to the top surface of said SEP.

Claim 48 (original): A novel enhanced SEP product, produced by the process of claim

41, effective to transport inorganic matter attached to the top surface of said SEP.

Claim 49 (original): A novel enhanced SEP product, produced by the process of claim

41, wherein said SEP is effective for use in conjunction with numerous other novel enhanced

SEP's for massive simultaneous tracking of material bound to the top surface of said SEP's as

the SEP's are pooled and put through at least one of a simple reaction and a series of pooled

reactions.

Claim 50 (original): A method for generating shape encoded response classes for use in

subsequent assays, comprising the steps of:

providing a multiplicity of novel enhanced SEP's;

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attaching probes to each of the multiplicity of SEP's;

reacting the multiplicity of SEP's having bound probes against a sample;

physically sorting the resulting products into at least two classes using respective

strengths of reporter signals; and,

decoding the results by automated shape imaging by class.

Claim 51 (original): The method of claim 50, wherein the providing step further comprises:

fabricating bulk quantities of SEP's.

Claim 52 (original): The method of claim 11, wherein the attaching step further comprises undergoing an attachment reaction to form SEP-probe conjugates.

Claim 53 (original): The method of claim 52, wherein the reacting step further comprises pooling the SEP-probe conjugates and dispensing randomly sampled aliquots.

Claim 54 (original): The method of claim 53, wherein the sorting step further comprises performing individual assays.

Claim 55 (original): The method of claim 54, wherein the decoding step further comprises using an automated imaging system.

Claim 56 (original): The method according to claim 55, wherein the decoding step further comprises using shape recognition software to generate a desired form and format for a resulting data set.

Claim 57 (currently amended): The method of claim 57 56, wherein massively multiplexing assays are accomplished using fewer spatial limitations and mixing constraints relative to existing methods of multiplexing probes.

Claim 58 (original): The process of claim 41, wherein the fabricating step further comprises maintaining a special free region optimal for probe attachment and detection on the top surface of said SEP, further reducing a likelihood of artifactual debris masking the unique geograph features imaged.

Claim 59 (original): A product, produced by the process of claim 58, which includes an addressable microreactor comprised of an encoded microwell.

Claim 60 (original): The process of claim 41, further comprising one-bit SEP imaging.

Claim 61 (original): In a generally planar shape encoded particle (SEP) made from a geometric base shape for arbitrarily large capacity and unambiguous multiplexed decoding, the improvement which comprises:

an adjustable coding capacity defined by a number of independent notches extending along a peripheral edge surface of the SEP whereby N notches encode for 2^N shapes.

Claim 62 (original): The SEP of claim 61, wherein the systematic notch patterns are defined by a following algorithm ("notching equation"):

i.
$$S[C](t) = b_0(t) + c_1 b_1(t) + c_2 b_2(t) + ... + c_N b_N(t)$$

ii. given a code word consisting of N digits in base k,

$$C = (c_1,c_2,\ldots,c_N),$$

iii. where c_i ε {0,1,...,k-1} (e.g., k=2 is binary code, each c_i is 0 or 1), it is associated with a 2-dimensional closed curve S[C]—the "shape"—defined by the "notching equation"

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iv. where $t \in [0,1]$ is a parameterization of the base shape boundary curve.

Here, $b_0(t)$ represents the equation of the base shape boundary, and the other $b_i(t)$ represent the equations of the basis of curves corresponding to the distinct potential "notches". The number of distinct code words, C, is k^N (2^N for binary coding).

Claim 63 (original): The SEP of claim 62, further comprising being distinguishable in any orientation by having at least an indicum to differentiate the spatial orientation of the SEP in terms of the relative position of the SEP upon at least one of a substrate upon which it is disposed and a material in which it is embedded.

Claim 64 (currently amended): The SEP of claim 63, wherein the image of S[C] is the boundary of a connected open set, further defined as the SEP is capable of being repeat manufacturable in that if the shape was cut from a material sheet, the SEP is substantilaly substantially free of dangling or disjoint parts interfering with the ability to distinguish the shape of the SEP.

Claim 65 (original): The SEP of claim 64, wherein a perturbing basis b_i(t) corresponds to isolated notches positioned about the peripheral edge of the geometric base shape.

Claim 66 (original): The SEP of claim 65, wherein said base shape is a square; and the isolated notches further comprise completely disjoint smaller square notches.

Claim 67 (original): The SEP of claim 64, wherein a perturbing basis bi(t) corresponds to distinct frequencies of sinusoidal ripples defined by distinct Fourier Modes along the boundary of a circular base geometric shape.

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Claim 68 (original): The SEP of claim 64, wherein a perturbing basis bi(t) corresponds to the first N nodes of a wavelet basis, defined by positional frequencies having specific mathematical properties for curves along the geometric base shape perimeter.

Claim 69 (original): The SEP of claim 61, further comprising a multiresolution (fractal) curve defined by a following algorithm:

- $S[C_{i+1}](t) = S[C_i](t) + c_1^i b_1^i(t) + c_2^i b_2^i(t) + ... + c_{N_i}^i b_{N_i}^i(t)$
- ii. given any series of M base k code words of the type above, X = $\{C_1,...,C_i,...,C_M\}$, the single shape defined by this entire series, S[X], is defined iteratively as follows: the first iteration, S[C₁], is defined as usual above, relative to some base shape;
- where $S[C_i]$ plays the role of the base shape for this step, and $b^i_{\ m}$ is a basis iii. of perturbations along this new base shape. These will typically be a reduced scale form of the type of notches used on the coarsest scale. The final or limiting shape produced by this process, $S[C_M]$, is the shape S[X]. The number of encoding shapes described this way is the number of encoding vectors, X, which is k, where N is now the total number of encoding digits in X, $N = N_1 + N_2 + ... + N_M$.

Claim 70 (original): The SEP of claim 69, further comprising being distinguishable in any orientation by having at least an indicum to differentiate the spatial orientation of the SEP in terms of the relative position of the SEP upon at least one of a substrate upon which it is disposed and a material in which it is embedded.

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Claim 71 (original): The SEP of claim 70, wherein the image of S[C] is the boundary of

a connected open set, further defined as the SEP is capable of being repeat manufacturable in

that if the shape was cut from a material sheet, the SEP is substantially free of dangling or

disjoint parts interfering with the ability to distinguish the shape of the SEP.

Claim 72 (original): The SEP of claim 64, further defined by a concise scalable and

general specification language comprising a definitive size for the geometric base shape and a

plurality of basis coefficients further describing the shape.

Claim 73 (original): A process for designing a photolithography mask for high density

fabrication of SEP's, comprising the steps of:

choosing a standard polygon which polygon surrounds a desired shape

circumferentially:

replicating the standard polygon in a high density closed space regular array for

defining available shape positions by covering the available mask area with N polygons; and

populating available locations on the mask given a set of N desired shapes.

Claim 74 (original): The process of claim 73, implemented in software further

comprising the steps of:

inputting a mask size, a standard polygon size and a specification history listing of

desired N shapes;

translating the description into mask pattern specifications; and

automatically producing as output a mask specification file suitable for reading a

pattern generator system used to fabricate the mask.

Claim 75 (original): The process of claim 74, further comprising the steps of:

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placing the N desired shapes on a mask for a single wafer;

etching the single wafer;

releasing the entire set of shapes; and

collecting the entire set of shapes into a single pool.

Claim 76 (original): The process of claim 73, further comprising the steps of:

using the resulting mask to perform reactive-ion-etching of a silicon-on-insulator

wafer; by,

reactive ion etching for sufficient time to cut through to the substrate;

subdividing the etched wafer into sub-sections for subsequent handling;

physically releasing the etched poly-crystalline silicon by dissolving the substrate

with a hydrofluoric acid treatment;

creating a resulting pool of freed SEP's;

surface treating the freed particles with at least one of heating and hydrogen

peroxide treatment to prepare the SEP's for subsequent handling; and,

storing the finished SEP's in purified H₂0.

Claim 77 (original): The SEP of claim 66, consisting essentially of a flake derived from

a wafer of silicon.

Claim 78 (cancelled)

Claim 79 (original): The SEP of claim 66, further comprising a magnetic material

selected from the group consisting of magnetic, ferromagnetic and magnetizable components.

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Claim 80 (original): A method of using shape encoded particles (SEP's) for DNA

analysis, which comprises the steps of:

providing a multiplicity of SEP's having distinct and imageable shapes;

binding each of said multiplicity of SEP's having distinct shapes to a desired

number of biological probes;

hybridizing the resulting combinations in solution; and

acquiring data in the form of reporter signals from the bound material.

Claim 81 (original): The method of claim 80, said providing step further comprising

each of the multiplicity of SEP's having the inherent ability to both identify material bound to

them and to track history of exposure as processing reaction steps alter their respective states or

the state of organic probes bound to them.

Claim 82 (original): The process of claim 38, said centralized probe attachment zone

further comprising at least an encoded microwell having a diameter I ranging from at least about

1 micron to 100 microns and a volume less than or equal to 1 nano liter.

Claim 83 (original): A product, produced by the process of claim 82.

Claim 84 (currently amended): A genomic analysis method, using the product of claim

82, comprising the steps of:

coupling biological probes to the novel enhanced SEP's having microwells;

mixing all of the novel enhanced SEP's having microwells together;

loading reaction mixutures mixtures into the microwells by soaking;

sealing the reaction wells;

and monitoring the reaction.

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Claim 85 (original): The method of claim 84, further comprising:

transferring the loaded particles from their aqueous loading solution into oil,

forming a tension seal of the microwells;

transferring the aqueous loading solution and particles onto a soft polymer

support surface, and impressing them into the surface with sufficient force based up the

centrifuge acceleration to form a pressure seal of those microwells facing downward into the

polymer;

transferring the aqueous loading solution and particles onto a support surface and

impressing upon them a compliant polymer sheet with adequate force to form a pressure seal on

those microwells facing upward into the sheet; and

undergoing a capping reaction whereby a multiplicity of spherical particles are

introduced into the aqueous loading solution having a predetermined size and surface coating for

fitting the multiplicity into the microwell openings and seal the microwell openings.

Claim 86 (original): The method of claim of claim 85, further comprising Polymerase

Chain Reaction (PCR) detection of DNA and RNA.

Claim 87 (original): The method of claim 86, the PCR being used for DNA analysis

whereby the shape encoded microwells are preloaded with PCR detection primers bound to the

microwell walls having different shapes carrying different detection primers, and the pool of the

microwell particles is introduced into the DNA sample to be analyzed, loaded by soaking and

then sealed, reacted with PCR and imaged for a reporter signal to detect and quantify whether the

subject DNA sample contains fragments corresponding to the involved detection primers.

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Claim 88 (original): The method of claim 87, further comprising using the reporter

signal to for biodefense monitoring by simultaneously screening a sample for DNA from a

multiplicity of pathogenic microorganisms and different strains of particularized pathogens.

Claim 89 (original): The method of claim 85, further comprising Enzyme Linked

Immunosorbant Assay (ELISA) detection of proteins.

Claim 90 (original): The method of claim 89, whereby the shape encoded microwells

are preloaded with ELISA detection antibodies bound to the microwell walls having different

shapes containing different detection antibodies for different proteins, and the pool of the

microwells is introduced into the protein sample to be analyzed in solution, loaded by soaking,

and sealed, reacted and imaged for a reporter signal to detect and quantify whether the sample

contains proteins corresponding to subject detection antibodies.

Claim 91 (original): The method of claim 90, further comprising using the reporter

signal to for biodefense monitoring by simultaneously screening a sample for DNA from a

multiplicity of pathogenic microorganisms and different strains of particularized pathogens.

Claim 92 (original): The genomic analysis method of claim 85, the monitoring step

further comprising using a fluorescent microscope or scanner to detect at least one of a positive

signal (reaction) and a negative signal (no reaction).

Claim 93 (original): A method of use of the SEP of claim 61, as an embedded

identification device for bulk materials, whereby a multiplicity of said SEP's are at least one of

surface coated onto workpieces; embedded into solid materials during manufacturing; mixed into

powdered materials; and suspended in liquid or gas phase materials.

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Claim 94 (original): A method of use of the SEP of claim 62, as an embedded

identification device for bulk materials, whereby a multiplicity of said SEP's are at least one of

surface coated onto workpieces; embedded into solid materials during manufacturing; mixed into

powdered materials; and suspended in liquid or gas phase materials.

Claim 95 (original): A method of use of the SEP of claim 61, as an embedded

identification device for military, police, criminal or terrorist activities by rendering specialty

plastic, ceramic and metallic items traceable to their owners, manufacturers, or distributors.

Claim 96 (original): The method of claim 95, further comprising emplacing SEP's

within explosive devices.

Claim 97 (original): A method of use of the SEP of claim 61, as an embedded

identification device for high value components subject to theft.

Claim 98 (original): A method of use of the SEP of claim 61, as an embedded

identification device for sample tracking in forensic or biomedical analyses.

Claim 99 (original): A method of use of the SEP of claim 61, as an embedded

identification device for quality control in mixture based procedures.

Claim 100 (original): A method of use of the SEP of claim 61, as an embedded

identification device for materials released into the environment.

Claim 101 (original): A method of use of the SEP of claim 61, as an embedded

identification device for rapid screening of different trial mixtures having a desirable property or

ingredient.

Claim 102 (original): A method of use of novel enhanced SEP's comprising the steps

of:

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generating a library of possible combinations of an identifiable set of molecular

building blocks;

testing the molecular building blocks for a set of desired properties;

storing results on a number of N SEP's.

Claim 103 (original): The method of claim 102, further comprising providing a stem

cell line, cultured onto the N shape encoded particles a tracking assay from the to screen a large

number of treatment series corresponding to exposure to or withdrawal of different growth

factors and measuring by a reporter for a particular cell type.

Claim 104 (currently amended): A shape encoded particle (SEP) based combinatorial

synthesis process, comprising the steps of:

providing a multiplicity multiplicity of novel enchanced enhanced SEP's which

are effective for two-dimensional image creation over a massive number of possible

combinations of geometric shapes by enabling display of a definitive outline when imaged by a

suitable technique;

synthesizing directly onto the SEP's a predetermined range of combinations of

chemical moieties and entities to be systematically tested for functionality;

whereby an encoded screening assay is created of the directly synthesized

combinations of chemical moieties and entities such that a library of the same is created.

Claim 105 (original): The process of claim 104, wherein an SEP combinatorial

treatment tracking assay is generated by a series of steps, comprising, in combination:

starting with a series of cells;

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tracking the response of the cells to a predetermined series of possible sequential

treatments by a set of factors, wherein the order of application is important;

archiving the resulting data by SEP information; and

repeating the process X times as needed.

Claim 106 (currently amended): The process of claim 105, the tracking step further

comprising:

carrying out a predetermined universe of possible combinations of treatment on a

material;

recording the outcome of the possible treatment series;

dividing dividing a particle pool of resulting outcomes onto N distinct SEP shapes;

pooling the resulting SEP's;

arbitrarily dividing the SEP's into a plurality of treatment sub-groupings;

imaging the sub-groupings in such a way that the outcome is accociated

associated with the shapew shape of each particle.

Claim 107 (currently amended): The process of claim 106, the earrying out

step further comprising:

providing a set of chemical building blocks;

linking the building blocks to form a k-mer, or string of K linked blocks;

preparing a pool of N distinct SEP shapes;

undertaking a derivatizing reaction on the pool to prepare the SEP particles

surfaces;

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dividing the SEP pool randomly into M equal sub-pools:

iamging eahc sub-pools imaging each sub-pool to record which shapes are

included; and

repeating the process for K times.

Claim 108 (currently amended): A product produced by the process of claim 107, which is a shape encoded library.

Claim 109 (currently amended): The process of claim 105, the an SEP combinatorial treatment tracking assay is effective for producing a library of serially treated materials or an assay for a treatment series producing a design effect on the starting material.

Claim 110 (original): A method of use of novel enhanced shape encoded particles (SEP's) for structural geometric forensic reconstruction comprising the steps of:

creating a pool of N distinct SEP's, from a single etching process;

applying the SEP's to the surface of a completed structure;

imaging the entire structure in a series of shape recording images mapped in three ordinal planes onto the structure; and,

retracing the structure from a fragment following a partially destructive event.

Claim 111 (currently amended): A method of use novel enhanced SEP's for seeding cultured pearls, comprising the steps of:

emplacing an SEP as a seed particles with a pearl culturing medium in the place of a standard seeding particle such as an oyster shell spherule;

recording the encoded identity of the SEP; and,

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imaging the SEP non-destructively with x rays;

for at least one of authentication, tracing, theft prevention and reclamation.